BIOREMEDIATION OF CADMIUM USING MICROBE

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I declare that this thesis entitled “Bioremediation of Cadmium Using Microbe” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree

Signature : ……..

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Date : 16 MAY 2008

To my beloved father and mother

Tn Hj Elias B. Zakaria

Pn Hjh Siti Rajmah Bt Hj Ariffin

And my dearest sisters and brother.

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ABSTRACT

Bioremediation is a technique that can be used to clean up the contaminated site or water. This method is advancement in biotechnology development of biological method in removal and contaminant degradation. By doing this research, we can study how it can solve the contaminated site and water problem. The main objective of this research is to study the potential microbe to remove cadmium by using bioremediation method and also to characterize the bacterial that exist from wastewater contained cadmium. The sources of contaminated water that contain cadmium was collected from wastewater plant located at Huntsman Tioxide (M) Sdn Bhd, Kemaman Terengganu Darul Iman. In this research, there will be a several methods used such as isolation, characterization and degradation of cadmium that can be used in bioremediation techniques. The isolated microbe from wastewater sample which is P1 is gram-negative bacterium and have cocci in shaped. It exhibited at the greatest cadmium tolerance. It was able to survive in 10mg/l of cadmium concentration. In culture exposed to 10mg/l, microbe P1 cells degrade 74.9% of cadmium in first day and decreased at second day to fifth day; 19.9%, 0.1%, 0.6% and 2.3% respectively. The highest amount of cadmium removed per ml of culture was observed in first day with 7.49 mg/l of cadmium. In conclusion, the microbe P1 has been shown the great potential of degrading the cadmium inside the wastewater.

ABSTRAK

Bioremediasi adalah satu kaedah yang boleh digunakan untuk membersihkan kawasan atau air yang tercemar. Kaedah ini adalah merupakan perkembangan teknologibio yang menggunakan kaedah penyingkiran biologi dalam menyingkirkan dan pembersihan bahan tercemar. Daripada kajian ini ,kita akan dapati , bagaimana masalah pencemaran air dan kawasan dapat diselesaikan. Tujuan utama kajian ini dilakasanakan adalah untuk mengkaji potensi mikrob untuk menyingkirkan cadmium dengan menggunakan teknik bioremediasi dan juga untuk mengkaji bakteria yang terhasil daripada air tercemar yang mengandungi cadmium. Sumber air tercemar ini di ambil dari Huntsman Tioxide (M) Sdn Bhd, Kemaman Terengganu Darul Iman. Didalam kajian ini, terdapat beberapa kaedah seperti pemisahan, pengelasan dan penyingkiran cadmium menggunakan kaedah bioremediasi. Bakteria yang telah disingkirkan dari sampel air buangan, mikrob P1 adalah merupakan bakteria jenis *Gram Negative* dan berbentuk sfera; di namakan microb P1 yang mana dapat menyingkirkan sejumlah besar kandungan cadmium. Ia dapat hidup di dalam 10 mg/l. Sel mikrob P1 dapat menyingkirkan 74.9% cadmium pada hari pertama dan menurun pada hari kedua sehingga hari kelima sebanyak 19.9%, 0.1%, 0.6% dan 2.3 % mengikut turutan. Kepekatan tertinggi cadmium yang dapat di singkirkan per ml daripada kultur telah di perhatikan pada hari pertama adalah sebanyak 7.49 mg/l. Secara kesimpulannya, mikrob P1 telah menunjukkan potensi terbaik dalam menyingkirkan cadmium dalam air terbuang.

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LIST OF SYMBOLS

µ	-	micro
°C	-	degree Celsius
ml	-	millimeter
mg/l	-	milligram per liter
sec	-	second
°	-	degree
h	-	hour
t	-	time

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CHAPTER 1

INTRODUCTION

1.1 Background of studies

Our environment consists of very kinds of natural and artificial metal. Nowadays, metal played as large role in industrial development and technological advance. Some of the metals used in industrial field are light metal and some more is heavy metal. The term ‘heavy metal’ is the natural component of earth crust which cannot be damaged or degraded. In hindsight, this group should preferable referred to as ‘toxic elements’. All of these elements are included in the UEPA’s as a very hazardous element in pollutant (SenGupta., 2002).

Metal has classified as essential, beneficial and detrimental. Trace elements recognized as essential for human body consist of iron, zinc, copper, chromium and molybdenum in small scale but it will become inhibitory to toxic with an increase in concentration and lead to poisoning.

Large quantities of organic and inorganic compound are release into environment every year as a result of human activities. These phenomenon come from industrial activities such as mining, oil refinery and agriculture, which had been done through out the world. Increasing environmental pollution by heavy metals results from their increasing utilization in industrial processes (Nriagu and Pacyna, 1988) the activities not only may affect the environment disaster, but it can cause harmful to living organisms.

Early year 2006, statistics released by the Department of Environment of Malaysia have shown that out of the 120 river basins having 926 monitoring stations, it was found that 44.5% of the stations show the water was in clean category, 48.4% slightly polluted and 7.1% polluted. In terms of number of river basins, 58 river basins were clean, 53 were slightly polluted and 9 were polluted. The water quality data also revealed that 13% of the river basins being monitored were polluted by waste and effluent discharged from agriculture-based and manufacturing industries, 24% by the effluent from livestock production, 23% by earthworks and the remainder by other sources. Sediments of the Juru and Langat rivers are contaminated by Pb and Zn, while the Langat River was heavily contaminated by Cd. The concentration of Zn and Pb in coastal sediment off Juru in Penang and in Johor Strait were two and three times higher than global shale value (Azhar *et al.*, 2006). All these statistic need to be taken seriously because this is our responsibilities to take care of water cleanliness and quality.

Bioremediation technique is about remediation of contaminated sites using either microorganisms or plants to detoxify the site largely by transforming or degrading the pollution (Bhattacharya *et al.*, 2002). In simple word, bioremediation is one method to treat the environment or polluted area using living cell such as *microbe* or using plant such as *microalgae*. We used this technique because bioremediation technique cannot 4give side impact after applying the method. Not such chemical technique will give harmful effect to the earth. In addition, bioremediation is a well-established technology for the removal of organic soil contaminants (Bhattacharya *et al.*, 2002). The process generally operate under condition that enhance the activities of either the native microorganism or the introduced species, particularly for those elements that transform or degrade under condition suitable for optimum performance of the microorganism. There are many advantages to the use of Bioremediation for treatment of hazardous waste sites (Alexander, 1994):

i. Can be done in situ
ii. Keeps disruption to a minimum
iii. Eliminate transportation costs and liabilities
iv. Eliminates waste permanently

v. Eliminates long-term liability
vi. Biological systems are often less expensive than conventional treatment
vii. Can be coupled with other treatment techniques to form a treatment train

1.2 Problem statement

Higher amount of heavy metals that contaminate inside food, drinking water or air would give dangerous effect to human body. These heavy metals such as cadmium, chromium and mercury could cause hazardous illness such as kidney spoilage, skin lesions, fatigue lung and increased blood pressure. Mostly, these are the general effects that occur if someone has higher amounts of heavy metals in their body. Clinical diseases may occur as a result of ingestion of high doses of soluble selenium salt. Such as poisoning cases display nausea, vomiting and subsequently hair and nail changes and skin lesions (Nordberg *et al* 2002). Person ingesting large doses of copper in addition to vomiting, nausea and diarrhea may develop hematuria and jaundice (Nordberg *et al* 2002). Excessive doses of soluble salts of iron or copper by the oral route, which may be ingested by accident or with suicide intent, give rise to extensive and severe gastrointestinal manifestation, systemic toxicity, shock, and lethal effect (Nordberg *et al* 2002). These are several affects from heavy metal if it release freely to human body and environment.

1.3 Objective

The main objective is to study the potential microbe which can remove cadmium by using bioremediation method and also to characterize the bacterium that exist from wastewater contain of heavy metal.

1.4 Scope of study

According to the objective stated. The scopes of research are:

i. To isolate cadmium tolerant bacterium
ii. To screen cadmium- degrading bacterium.
iii. To identify and characterized cadmium degrading bacterium.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Water Quality in Malaysia

The uptrend of urbanization, globalization and industrialization may contribute to water pollution. River and stream sustain many human usages including water supply for domestic usage, industrial site, recreational purpose such as swimming and fishing, transportation and many more. The quality of water is directly affected by the amount of material discharged into the river. Industries site and urban dweller has contribute large number of water pollution in Malaysia. Water pollution seriously may affect human life very hard to get quality water which is beneficial for daily usage. It endangers human health, may cause shorten the average life of the country population and also may effect destroy of aquatic live and it biodiversities.

Early year 2006, there have been two cases of water supply contaminations occurring in the state of Selangor. The Sungai Selangor which provides water supply to the northern part of Klang Valley was contaminated for a number of days due to the overspill of leachate from a nearby solid waste dumping area. The strong smell of the water itself, believed to be caused by ammonia, indicates that it is clearly unsafe for any use especially for drinking purpose. Sungai Langat which provides water supply for the southern part of Klang Valley has posed the same problem at about the same period where the improper management of solid waste dumping site again has become the culprit (Keizrul, 2006). These entire situations give bad impact in human life. People

feel not secure to use the water. It happened because of water quality nowadays not guarantee it cleanliness.

Formerly, water pollution phenomenon happen because of overspill the waste which is include unwanted material inside water such as heavy metal. This has led to increasing concern about the effects of toxic heavy metals as environmental contaminants. (Zakaria,. 2001) This is material may caused water to be hazardous to human and environment

2.2 Bacteria

Bacteria are the simplest life form and are the most various organisms with respect to number of species and total biomass. They are small, unicellular prokaryotic organism. Bacteria are classified by it morphology, response to chemical stains, nutrition and also it metabolism.

2.2.1 Morphology and classification

Most bacteria are very small, almost are micrometers μm (10^{-6} meters) in length. The size is microorganism may be described according to their length and width. For example *Escherichia coli,* a common bacterium found in human faces and wastewater treatment plants, is approximately 2 μm in length and 0.5 μm in width (Gerardi., 2006).

Most bacteria can be grouped into variety of shapes and sizes; it called the morphology of the organism. The most common shapes are rod-like bacillus (plural, bacilli) form or spherical form called coccus (plural, bacilli). The rod forms vary considerable from very short rods that almost look like cocci, to very long filaments thousands of microns in length. Bacteria also form spirals and corkscrews, oval

(coccoid), commas and elaborately branches structures. The cocci often take on multi-cell forms, as two cocci joined together it may called as diploccoci, a chain of cocci known as streptococci or if consist of four cell in cube, it may call as tetrads (Science in the Real World.,1999). Figure 2.1 below, show several shapes and arrangement of bacteria.

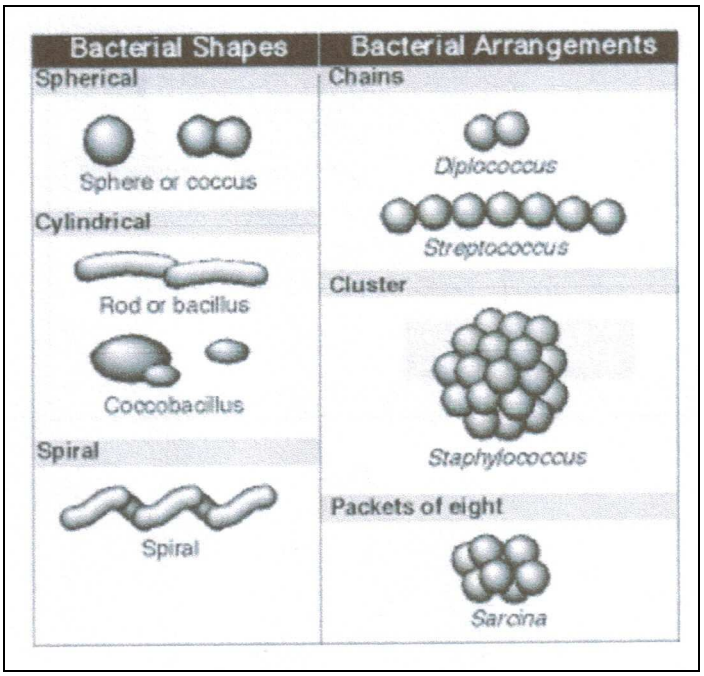

Figure 2.1: Bacterial morphology (Science in a real world, 1999)

A second major of bacterial criterion for distinguishing the bacteria is based on it cell wall structure. There are types of cell wall that give different staining characteristic with series staining of reagents called the gram stain. Bacteria with thin wall layer and outer membrane remain pink color, there are called gram negative bacteria and bacteria with thicker wall layer, also lacking the outer membrane called as gram positive bacteria. Table 2.1 below shows the difference between gram negative and gram positive bacteria

Table 2.1: Significant differences Between Gram negative and Gram Positives bacteria (Gerardi., 2006)

Characteristic	Gram Negative Bacteria	Gram Positive bacteria
Lipids	Much lypopolysaccharide	Very little
Peptidoglycan	Thin layer	Thick layer
Outer membrane	Present	Absent

2.2.2 Bacterial growth curve

Bacterial growth can be plotted through it growth curve. The graph consist of number of cell versus time of growth. The cell number is plotted as the log of cell number, since it is an exponential function. Regardless of the generation time, in growing culture the plot of the log cell number versus time give a characteristic curve. This curve typically has four distinct phases start by lag phase, exponential phase, stationary phase and death phase. Figure 2.2 shows the bacterial growth curves.

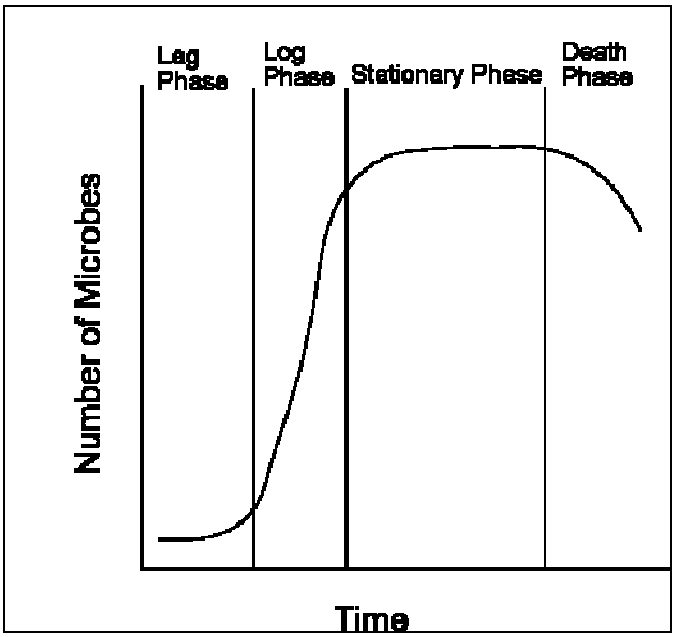

Figure 2.2: Bacterial growth curve (sources: http://www.eng.auburn.)

The lag phase is the first stage of bacterial growth. It is characterized by no increase in cell number, however the cell are actively metabolizing in preparing for cell division. This phase is depending on the growth medium, the lag phase may be taking a very short or very long time. Once the cell are actively metabolizing they begin DNA replication and shortly after the cell divide. These begin the second stage of growth called exponential phase or log phase of growth. This period, the cell grows rapidly, doubling at a fairly constant rate. The time takes the culture to double is called generation time.

The third phase in the growth of bacteria is stationary phase, when metabolism slows and the cells cease rapid cell division. They may divide slowly for a time, but soon stop dividing completely. There are still alive and maintain a slow metabolic activity. The factors that cause cells to enter stationary phase are related to changes in the environment, typically caused by high cell density. Among the changes that slow growth are depletion of nutrient and accumulation of waste products.

The final phase of the growth cycle is the death phase. In this phase the cell quickly lose the ability to divide even if they are placed in fresh medium. Like the phase of rapid growth the death phase also exponential; therefore cell die quickly and within hours a culture may have no living cells. The death phase and in fact all the phases can be slowed by lowering the temperature. Hence, in order to maintain the maximum cell viability it is best to grow bacterial culture only to early stationary phase and then chill the culture. So that, the bacterial will be still alive in much long period.

2.2.3 Factor of growth effect

Microorganism need nutrients to stay alive same as others living creatures. There are numerous environmental factors to ensure the microorganism is continuously can survive. There are including temperature, pH, moisture content and energy sources that can supply to microorganism’s growth. While most bacteria grow best when these

parameters are optimum for that strain, in the real world microbes can expect frequent environmental changes. In fact some bacteria have evolved to thrive in environments that are inhospitable for most life.

Below are some explanations on this parameter that give environmental effects to microbes.

2.2.3.1 Temperature

Temperature plays an important role in controlling the nature and the amount of microbial metabolism. If the temperatures exceed the acceptable limit, enzyme will damage or denatured. If the temperatures decrease, reproduction will stop while the organism focuses its energy on continuing life. If the temperature drops too low, then the organism will stop sustaining it life. There are suitable temperatures for each microorganism and a range in which these organisms may survive (G Tchobannoglous *et al*, 2004)

According to the temperature profile in Table 2.1 which they function best, bacteria may classified as *psychrophilic*, *mesophilic* or *thermophilic.* (G Tchobannoglous *et al*, 2004)

Table 2.2: Temperature classification of biological process (G Tchobannoglous *et al*, 2004)

Type	Temperature range, °C	Optimum range, °C
Psychrophilic	10-30	12-18
Mesophilic	20-50	25-40
Thermophilic	35-75	55-65

2.2.3.2 pH

The pH of the environment and water are also main factors of selection, survival and growth of microorganisms. There is generally a small range of pH needed for microorganism is capable of sustaining life. If the water becomes very acidic or alkaline than the concentration of microorganism slowly reduce. Most of bacteria cannot tolerate to pH levels above 9.5 or below 4.0. Generally, the optimum pH for bacterial growth is between 6.5 and 7.5. (G Tchobannoglous *et al*, 2004)

2.2.3.3 Moisture content

A water resource is a requirement for microorganism life. For each microorganism there have optimal moisture content for it to survive and growth. Microbes are limited to soluble materials that are transported crossing their cell membranes into the interior of the cell. The moisture soluble the substrate and allows the substrate to enter the cell. For hydrocarbon contaminated soils, moisture level below 50% appear to inhibit degradation. (Cookson, 1995)

2.3 Heavy metal

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed to a small amount they enter our bodies via food, drinking water and air. As trace elements, some heavy metals are essential to maintain the metabolism of the human body. But at higher concentrations they may caused to poisoning.

Therefore, heavy metal can enter a water supply by industrial, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. All of these would bring a lot of environmental and health hazard to